Shotgun Femtosecond Diffractive Imaging of Free Nanoscale Biomaterials

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Femtosecond diffractive imaging is a new x-ray microscopy technique for imaging non-crystalline objects beyond the damage threshold. Part of a new era of X-ray science delivered by X-ray free electron lasers (FEL), this technique has the potential to deliver three-dimensional atomic resolution structures of non-crystalline nanoscale biomaterials such as single biomolecules using images collected from reproducible copies exposed to the beam one by one. Experimentally, container-less delivery of single biomolecules to the X-ray pulses is imperative because any atom present in the X-ray path will contribute to the diffraction pattern. Here we use the soft-X-ray FEL in Hamburg (FLASH) to perform the first demonstration femtosecond diffractive imaging of free nanoscale biomaterials via a shotgun approach-a continuously refreshed stream of single nanoparticles synthesized *in situ* at atmospheric pressure by charge-reduced electrospray of a sucrose solution containing megadalton DNA origami complexes. In our method, the aerosol is transformed into a tightly focused particle stream in-vacuum using a set of aerodynamic lenses and single events of the interception of individual nanoparticles with an intense 10 femtosecond pulse, containing $\sim 10^{12}$ photons at 13.5 nm, results in a coherent diffraction pattern captured with a single photon sensitive X-ray camera. Reconstructed images of intercepted particles are obtained by phase retrieval through oversampling. Ions generated from the explosion of the nanoparticle are detected by a miniature mass spectrometer, providing insights to chemical composition. In its current configuration shotgun femtosecond diffractive imaging operates at 0.1 Hz and is directly transferable to future hard-X-ray FEL sources.